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L4: Entry 1 of 5

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US-PAT-NO: 6156726

DOCUMENT-IDENTIFIER: US 6156726 A

TITLE: Voltage-gated calcium channel antagonist and method

DATE-ISSUED: December 5, 2000

INVENTOR-INFORMATION:

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US-CL-CURRENT: 514/12; 514/21, 530/300, 530/324, 530/858

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| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image |
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☐ 2. Document ID: US 5770447 A

L4: Entry 2 of 5

File: USPT

Jun 23, 1998

BSPR:

The 34893 2L cells are useful for developing clonal cell lines expressing calcium channels.

BSPR:

The 34893 2L cells are for the general expression of high voltage activated calcium channels of a variety of types including but not limited to N type channels, R type channels, Q type channels, and cardiac Class C, L type channels.

DEPR:

The antibiotic Hygromycin B sulfate was obtained from Boehringer Mannheim. cDNAs for the rabbit skeletal muscle calcium channel alpha 2 subunit, and the human neuronal beta 2 subunit cloned into the expression vector pcDNAIII were obtained from Neurex. Human neuronal calcium channel alpha 1 subunits of E class and B class, cloned into the expression vector pcDNAIII, were obtained from Neurex.

DEPR:

Cell lines that were expressing high levels of both alpha 2 and beta messenger RNA were expanded and used for subsequent transfections. The cells were maintained in selective medium. Transfections of the B class and E class alpha 1 subunits were accomplished essentially as above, but plasmid containing the gene for hygromycin resistance was co-transfected with the plasmids containing the cDNAs for the B and E class alpha 1 subunits. Selection was in medium containing G418 at a concentration of 600 $\mu\text{g/mL}$, and Hygromycin B sulfate at a concentration of 400 $\mu\text{g/mL}$. Cells were selected in this medium and characterized for expression of functional calcium channels by electrophysiological methods.

DEPR:

The 2 L cell line is expressing the highest levels of both the alpha 2 and the beta subunit RNAs, and it was chosen as the vehicle for transfecting in B and E class alpha 1 clones.

DEPR:

FIG. 3 shows a comparison of the currents from S3 cells (2L cells which have been transfected with the B class, or N type alpha 1) and 192C cells (2L cells transfected with the E class alpha 1). As can be seen, the cells express channels that are similar, but differ slightly in their activation kinetics, and inactivation kinetics.

DEPR:

FIG. 4 illustrates the utility of the 34893 2L cells in looking at the binding of Neurontin to the alpha 2 subunit of the calcium channel. The level of specific binding to the transfected cells is about 20 times higher than that seen in the parent, untransfected HEK 293 cells.

CLPV:

providing a vessel containing calcium channel subunits of 34893 2L cell line deposited under the Budapest Treaty as ATCC No. CRL-12108;

Indeed, some authors have speculated that it is not possible to generate a set of cells which are stably expressing calcium channels at high levels. (Brust P. F., et al., Neuropharmacology, 1993;32).

BSPR:

One utility of cells expressing the alpha 2 and beta subunits of the calcium channel is in the area of transient expression. Recent work has identified regions of the calcium channel to which the conopeptides bind. These analyses were performed in *Xenopus oocytes*. In this system one can only measure the rates at which the peptides bind to the channel (Ellinor P. T., Zhang J. F., Horne W. A., Tsien R. W. *Nature*, 1994;372). Transient expression of these alpha 1 subunits in cells that are expressing the alpha 2 and beta subunits would allow for equilibrium binding measurements to be performed, allowing for more complete evaluation of the interaction between the channel and the peptides.

BSPR:

WO 95/04822 teaches isolated cDNAs encoding each of human calcium channel alpha 1 to alpha 2, beta, and gamma subunits, including subunits that arise as splice variants of primary transcripts. In particular, DNA clones encoding each of the alpha 1A-1, alpha 1A-2, alpha 1E-1, alpha 1C-2, alpha 1E-3, beta 3-1, beta 2C, beta 2D, beta 2E, and beta 4 subunits of human calcium channels are provided.

BSPR:

U.S. Pat. No. 5,386,025 teaches calcium channel gamma subunit encoding cDNAs.

BSPR:

The instant invention of developing stable cell lines expressing calcium channels by generating cells in which two of the subunits are expressed at high levels and using the 2L cells to transfect in the alpha subunit for any calcium channel to obtain cells expressing a new calcium channel type is not taught by the references.

BSPR:

The purpose of the instant invention is the development of cell lines that allow the rapid development of cell lines that are stably expressing a variety of different calcium channels.

BSPR:

The instant invention overcomes the difficulty in developing stable cell lines expressing calcium channels which is due in large part to obtaining stable incorporation of three different subunit clones in a single cell line. By generating cells in which two of the three subunits are expressed at high levels, generation of cell lines that express high levels of channels has been greatly simplified. Now, using the 2L cells, one can simply transfect in the alpha 1 subunit for any of the different calcium channels and have cells expressing a new calcium channel type.

made up of alpha 1, alpha 2, and beta subunits.

BSPR:

Recent experiments have shown that there are a number of other calcium channels in the central nervous system. The P type channel has been described in cerebellar Purkinje cells. This channel is a high voltage activated channel, but it differs from the N and L types primarily in its insensitivity to either dihydropyridines or conotoxins. Instead, this channel is sensitive to the peptide toxin Aga IVa from the funnel web spider. Cerebellar granule cells express a high voltage activated channel that has been called R or Q and is insensitive to Aga IVa, the conotoxins MVIIA and GVIA, and dihydropyridines. It is sensitive to the conotoxin MVIIC (Birnbaumer L., et al., Neuron., 1994:13).

BSPR:

Molecular cloning of the channel subunits from skeletal muscle and brain have revealed a significant similarity between the various different calcium channel subtypes. The level of conservation between the alpha 1 subunits of the N and L types is quite high, and this subunit has been identified as the subunit through which calcium ions flow. Several isoforms of alpha 2 and beta subunit clones have also been isolated from neuronal and muscle tissue, though there has been no definite assignment of specific isoforms to a particular type of calcium channel. As yet, there is no definite assignment of the P/Q type to a cloned cDNA.

BSPR:

Expression experiments in Xenopus oocytes have demonstrated that in order to produce fully functional calcium channels, the alpha 1, alpha 2, and beta subunits must all be expressed. Absence of either the alpha 2 or beta subunit results in a nonfunctional channel, even though the alpha 1 subunit, through which ions flow, is fully expressed. Indeed, not only the ion flux through these channels but the pharmacological properties of the alpha 1 are different in the absence of the alpha 2 and beta subunits. Expression of an alpha 1 subunit with different beta subunits results in channels with different inactivation properties, indicating that the beta-alpha 1 interaction is important in regulating the functional properties of the channels.

BSPR:

Expression of calcium channels in mammalian cells has lagged behind expression in Xenopus oocytes in part because Xenopus oocyte expression is quite convenient and in part because stable expression in mammalian cells has proven difficult. Many properties of the channels can be analyzed by electrophysiological techniques in Xenopus oocytes, and some pharmacology can be done using this system. Mammalian expression would allow a better characterization of the binding properties of drugs and peptides. Yet it has proven difficult to generate stable cell lines; whether because of an intrinsic toxicity of the expressed channel, or the combinatorial problem of expressing three subunits simultaneously in a single cell.